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The dynamics of the expression of C/EBP mRNA in the adult rat liver lobulus qualifies it as a pericentral mRNA

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A hybridocytochemical approach has been applied to establish whether the gene for the C/EBP mRNA might be involved in the topographical regulation of gene expression in adult rat liver. To that end the spatial distribution of the mRNA of C/EBP has been compared to that of the mRNAs of glutamine synthetase (GS), phospho*enol*pyruvate carboxykinase (PEPCK) and glucokinase (GK) in normal adult livers, in livers from dexamethasone-treated animals and in livers from starved animals refed with glucose for 4 h. In normal rat liver, in situ hybridization with a probe for C/EBP mRNA revealed a low density of apparently homogeneously distributed grains, indicating low levels of C/EBP mRNA. In contrast, the livers of the experimentally-treated animals revealed a zonal distribution of the mRNA of C/EBP with the highest density of grains around the central venules. The dynamics of the pattern of expression of C/EBP mRNA are virtually identical to that of the GK mRNA. These data qualify C/EBP mRNA as a pericentral mRNA and suggest a role for the C/EBP protein in the topographical regulation of the expression of the GK mRNA.

C/EBP; Enzymic zonation; Transcriptional regulation; In situ hybridization; Rat liver

1. INTRODUCTION

A central problem in modern biology is to understand the mechanisms by which the spatial pattern of gene expression is realized. The zonation of gene expression in the adult mammalian liver [1] within microcirculatory units named lobules, provides us with an amenable model to address this issue. Evidence from hybridocytochemical analyses is accumulating that the major site of control of the zonation of gene expression is at the pretranslational level. In adult rat liver many genes are expressed in gradients, either comprising the entire porto-central distance or only part of it [2]. In the latter case, we speak of a compartment-type of gene expression if a gene can no longer be expressed in all hepatocytes, but is confined to a periportal- or pericentral zone of cells. Paradigms of this type of zonation are GS [3–5], that is exclusively localized in a small pericentral compartment and CPS, exclusively located in a large contiguous periportal compartment [4,5]. In a gradient-type of zonation, genes are expressed in all hepatocytes in a gradient either decreasing from the portal to the central vein (albumin [6], PEPCK [1,7,8]), or the other way around (α-feto-protein [6], GK [1,9,10]).

To understand the origin and dynamics of this zonation we need to know the cellular interactions that might contribute to, and the molecular events that account for the zonal pattern of gene expression. Gradients of oxygen, hormones and substrates imposed by the unidirectional flow of blood through the liver, cell–cell or cell–matrix interactions, as well as hepatic lobular architecture are believed to be important determinants in the establishments of the zonal pattern of gene expression in the mammalian liver. However, an unsolved issue as yet is the question to what extent these factors are involved in regulating the rate of gene expression and in regulating the pattern of gene expression?

Recently, the molecular analysis of liver-specific gene expression has advanced with the identification of several regulatory factors [11,12]. These findings raised the possibility that the different patterns of gene expression in the liver might result from different patterns of expression of the individual transcription factors. As a first attempt to test this possibility we have analyzed the dietary- and hormonally-regulated pattern of expression of the mRNA of C/EBP (CCAAT/enhancer binding protein) [11,13] in comparison with the expression of a number of other mRNAs that may serve as examples of the various types of zonation.

Abbreviations: C/EBP CCAAT/enhancer binding protein; CPS, carbamoylphosphate synthetase; GK, glucokinase; GS, glutamine synthetase; PEPCK, phospho*enol*pyruvate carboxykinase

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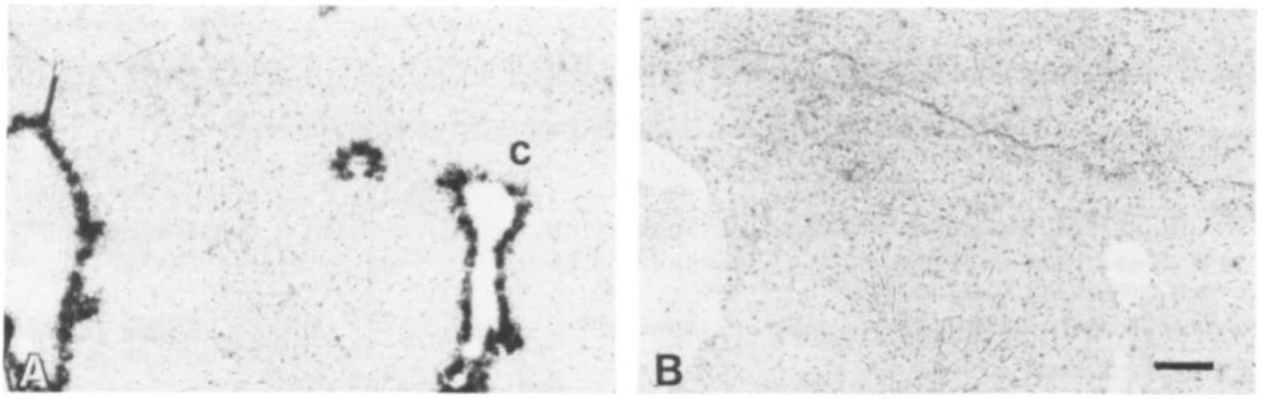

Fig. 1. Hybridocytochemical detection of the patterns of expression of the mRNAs of GS (A) and C/EBP (B) in the liver parenchyma of adult rat. c, central venule. Bar = 0.2 mm.

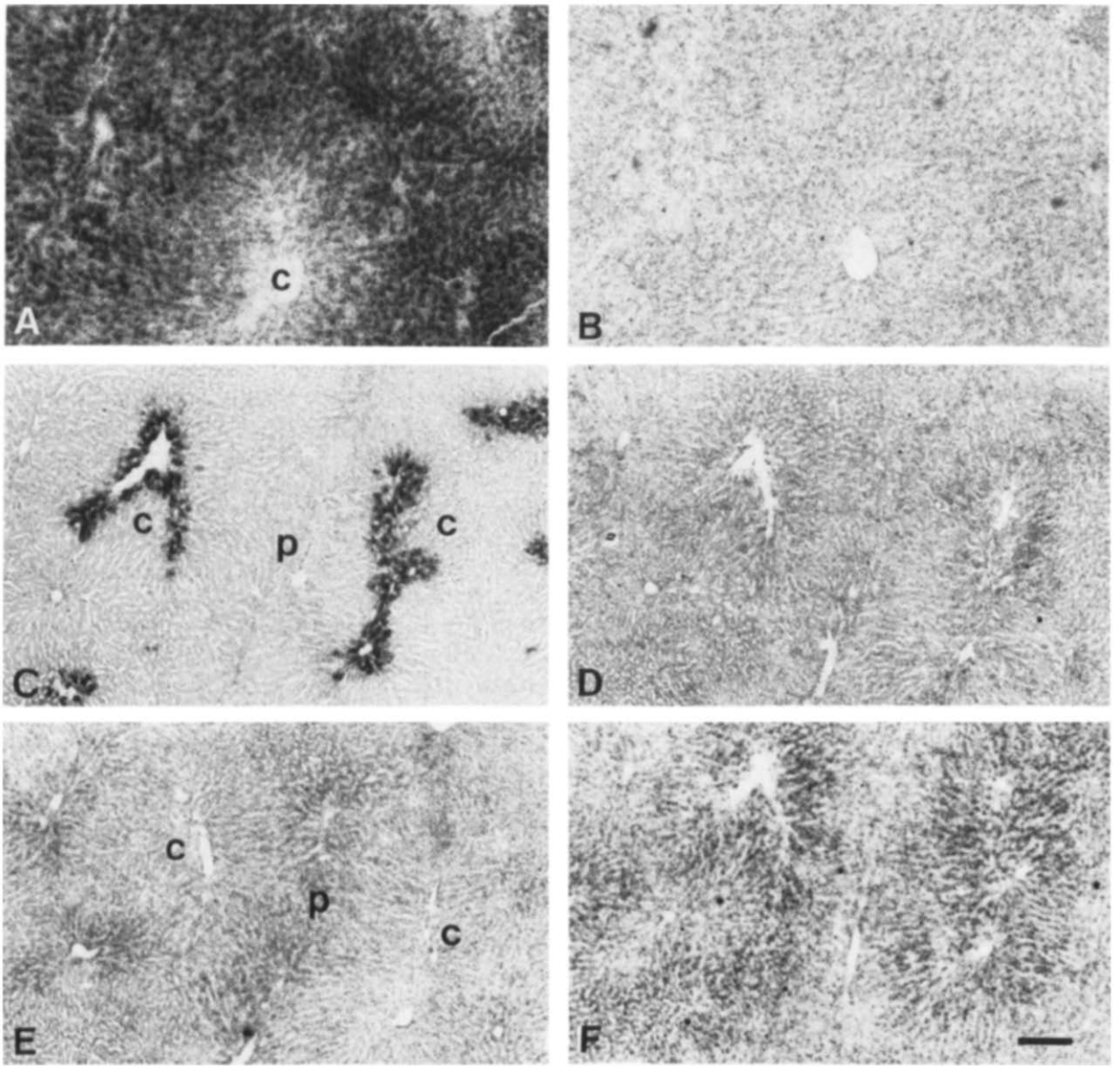

Fig. 2. Hybridocytochemical detection of the patterns of expression of the mRNAs of PEPCK (A,E), C/EBP (B,D), GS (C) and GK (F) in the liver parenchyma of adult starved rat (A,B) or adult starved rat, refed with glucose, as indicated in section 2 (C–F). p, portal tract; c, central venule. Bar = 0.2 mm.

2. MATERIAL AND METHODS

2.1. *Animals*

Wistar rats purchased from the TNO animal farm (Zeist, The Netherlands) were used. Starved animals were deprived from food for 48 h, refed twice, with a 2 h interval, by gavage with 5 g glucose per kg body weight and were sacrificed 2 h later. Glucocorticosteroid hormone (2.5 mg dexamethasone per kg body weight) was administered to animals that were fed at libitum 16 h before sacrifice. These animals and control animals were sacrificed at approx. 9.00 a.m.

2.2. *In situ hybridization, DNA manipulation and probe specifications*

Fixation, tissue processing and conditions of hybridization were described previously [10,14]. Autoradiography, using Ilford nuclear research emulsion (G5) was carried out at 4°C for 5 days. DNA fragments were purified from agarose gels (agarose, N.A., Pharmacia, Uppsala) and were labelled with [α-^{35}S]dCTP by random priming.

C/EBP mRNA was detected using a *NcoI-PstI* DNA fragment of clone pMSV-C/EBP-wt; this fragment contains the first 647 bp of the coding region of the rat gene [13,15]; the probe for GS mRNA was the 663 bp DNA insert of the GS3 cDNA subclone [16]; the 1081 bp *PstI* insert of the cDNA clone PCK-10 [17] was used for the detection of PEPCK mRNA and GK mRNA was detected using the 1800 bp *EcoRI* fragment of the pUC-GK1 cDNA clone [18].

3. RESULTS

In adult liver C/EBP mRNA is present at low levels [19,20] and has been reported to be homogeneously distributed across the liver lobule [21]. In agreement, hybridocytochemical analysis of the presence of C/EBP mRNA shows a low density of grains that are homogeneously distributed (Fig. 1B). The control section shows the normal distribution of GS mRNA in a small area, 1–3 cells wide, that lines the central venules (Fig. 1A), as reported previously [5]. PEPCK mRNA revealed a periportal pattern, and GK mRNA a homogeneous pattern of expression similar to previously published data [7,10,22] (not shown).

As the rate and pattern of expression of many typical liver genes is profoundly influenced by the dietary and hormonal state of the animal, we evaluated the pattern of expression of C/EBP mRNA under various metabolic conditions. It was found that starvation and diabetes (not shown), conditions that stimulate the expression of the periportally localized mRNA of PEPCK (Fig. 2A) and CPS [7,22], did not result in a significant change in the pattern of expression of C/EBP mRNA compared to the normal pattern (cf Fig. 1B and 2B). However, conditions that result in the pericentral induction of GK [10], display a concomitant pericentral induction of C/EBP mRNA. Four hours after an oral glucose load, a clear induction of C/EBP mRNA is evident (Fig. 2D). C/EBP mRNA is expressed in a definite gradient, decreasing in a central-portal direction. These conditions also lead to an induction of GK mRNA in the pericentral domain (Fig. 2F) [10]. PEPCK mRNA predominates in the periportal domain, albeit at relatively low levels (Fig. 2E) [7,8,22]. The expression of GS

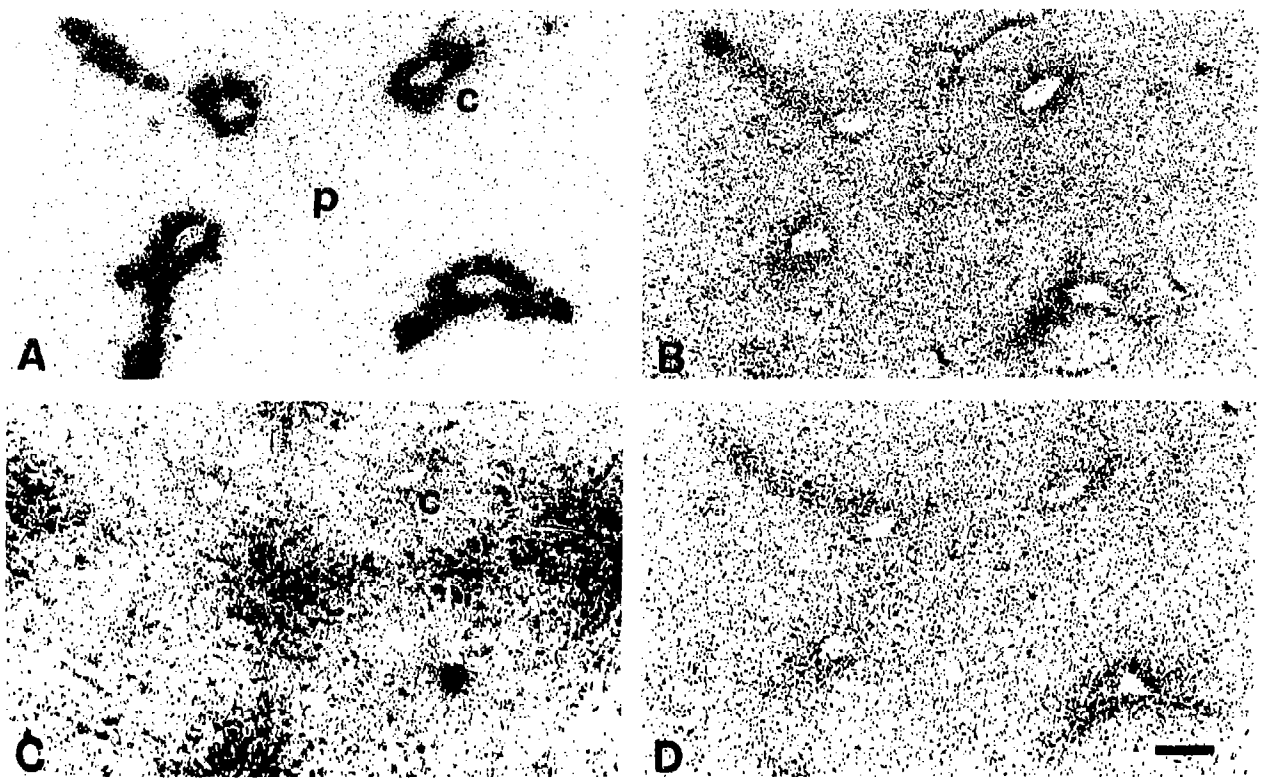

Fig. 3. Hybridocytochemical detection of the patterns of expression of the mRNAs of GS (A), C/EBP (B), PEPCK (C) and GK (D) in the liver parenchyma of adult dexamethasone-treated rat. p, portal tract; c, central venule. Bar ≈ 0.2 mm.

mRNA unambiguously identifies the small pericentral compartment around pericentral venules (Fig. 2C).

Recently we reported that the administration of dexamethasone to adult rats is able to induce GK mRNA in the pericentral domain [10] (Fig. 3D). Now we show that C/EBP mRNA is also pericentrally induced, in a virtually identical fashion (Fig. 3B). The patterns of expression of GS mRNA (Fig. 3A) and PEPCK mRNA (Fig. 3C) are essentially unchanged; GS mRNA being clearly induced [23], whereas the concentration of PEPCK mRNA is reduced, probably due to the glucocorticosteroid hormone-induced increase of insulin [24] as previously reported [10].

4. DISCUSSION

Several lines of evidence have designated transcription as the most frequently used and direct point of regulation of eukaryotic gene expression. The zonal expression of many liver-characteristic genes is no exception to this notion. This implies, as a necessary consequence, a zonal distribution of modulatory factors, such as transcription factors, hormones and/or their receptors, etc. It is an important question, which of the many factors that are able to modulate gene expression in the liver are particularly involved in the topographical aspects of this regulation? As a first attempt to address this question we have studied the spatial distribution of the mRNA of one of them, C/EBP.

The hybridocytochemical data presented demonstrate for the first time a zonal expression of the mRNA of a transcription factor in the liver lobule, suggesting that the protein encoded for is involved in the regulation of the zonation of gene expression in vivo. The pericentral pattern of C/EBP mRNA only becomes apparent under the inducing condition of glucocorticosteroids or under conditions that are known to induce the expression of glycolytic enzymes, i.e. glucose refeeding, and closely resembles the pattern of expression of the mRNA of the glycolytic enzyme GK. This demonstrates the value of in situ hybridization to trace factors potentially involved in the topographical regulation of gene expression in vivo, although it should be stressed that this approach discloses topographical correlations only.

C/EBP has been found to avidly recognize a disparate set of DNA sequences (CAAT/box motifs) in the promoter region of many liver-characteristic genes at sites that share only minimal sequence homology [11,25-28]. Moreover, several distinct CAAT/box-binding factors have been described (for a review see [11]). Computer analysis of the promoter region of the GK gene [29] could identify three CAAT/box motif homologs with the sequence TGTGGAAAG [30], the significance of which, however, remains as yet unaccounted for.

The intensively investigated promoter of the periportally expressed [6] gene for albumin contains six closely adjacent binding sites for nuclear proteins, among which one for LF-B1 (HNF-1) and three for C/EBP [31]. Using nuclear extracts from normal adult liver, LF-B1 has been demonstrated to play a dominant role in the transcription from this promoter [31]. Sofar, its distribution within the liver lobule is unknown. Use of nuclear liver extracts from animals of different metabolic states may shed light upon the significance of the other DNA-binding sites. For the time being we still have a great deal to learn from the plethora of possible combinations of modulatory factors. The delineation of the spatial expression of different modulatory factors may help to settle which of the factors might be involved in the regulation of zonal gene expression within the liver lobule.

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